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STEREOSPECIFIC CONVERSION OF DIOSGENIN TO LABELED a-ECDYSONE

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Summary: 15,16-[$^3H_2$]-α-Ecdysone has been synthesized from diosgenin.

We have previously reported [1] a stereospecific synthesis of α-ecdysone 1 from diosgenin 2. The physiologically important 22-R hydroxyl group was generated by stereospecific reduction of the diosgenin spiro-ketal group followed by reductive cleavage of the C-16-O bond. Due to the labile nature of various intermediates it was difficult to introduce tritium labels at C-15 and 16. In the following, we wish to report an alternative route which provides relatively easy access to [15,16-$^3H_2$]- and [26,27-$^{14}C_2$]-α-ecdysone.

Standard ketalization of 26-bromo-6-one 3 (prepared from 2 in 7 steps[1], crystals from MeOH, mp 147.5-148°) afforded the ketal 4, which was dehydrobrominated in refluxing DMF with LiBr and Li_2CO_3 to 6-ethylenedioxy-25-ene 5. Without LiBr no elimination occurred. Ozonolysis in CH_2Cl_2-MeOH deketalized 5, but a high yield of 6-ethylenedioxy-25-one 6 was obtained when 5 was ozonized in CH_2Cl_2 containing a small amount of pyridine followed by reduction with zinc powder and acetic acid at room temperature.

A fair yield of 26,27-bisnor-25-methoxycarbonyl compound 7 (crystals from MeOH-CH_2Cl_2, mp 194-195°) was obtained when 6 was subjected to bromoform reaction[2], esterification with diazomethane and reacetylation. A small amount of 24-acetoxy derivative of 7 was also formed apparently by overoxidation.

Bromination of 7 under equilibrating conditions produced a high yield of the corresponding 7α-bromide 8, which was dehydrobrominated to yield 7-en-6-one 9, mp 210-211° (MeOH-CH_2Cl_2), ms 530(M^+); pmr δ 5.69(brt,7-H); uv 243 nm (ε 13,300); cd $\Delta\varepsilon_{325}$ +4.52, $\Delta\varepsilon_{243}$ -18.6[3], as the major product accompanied by the corresponding 4-en-6-one, mp 162-163°(MeOH), ms 530(M^+), pmr δ 5.89 (dd,J=3 and 2Hz,4-H); uv 232 nm (ε 7,080); cd $\Delta\varepsilon_{319}$ -2.30, $\Delta\varepsilon_{233}$ -8.00.

Selenium dioxide hydroxylation of 9 afforded the expected 14α-hydroxy-7-en-6-one 10 mp 227-229° (MeOH), ms 546(M^+); pmr δ 5.86(d,J=3Hz,7-H); uv 239 nm (ε 11,900); cd $\Delta\varepsilon_{331}$ +3.42, $\Delta\varepsilon_{246}$ -9.56, which was converted without purification to the conjugated 7,14-dien-6-one 11, ms 528(M^+); pmr δ 6.15(d,J=3Hz,7-H), 5.79(d,J=3Hz,15-H), 4.72(dd,J=7 and 3Hz,16-H); uv 275 nm (ε 9,500), cd $\Delta\varepsilon_{272}$ -19.2; ir 1730, 1665cm^{-1}, upon treatment with trifluoroacetic anhydride and dry pyridine[4]. In the absence of selenium dioxide contaminant, the major product was the

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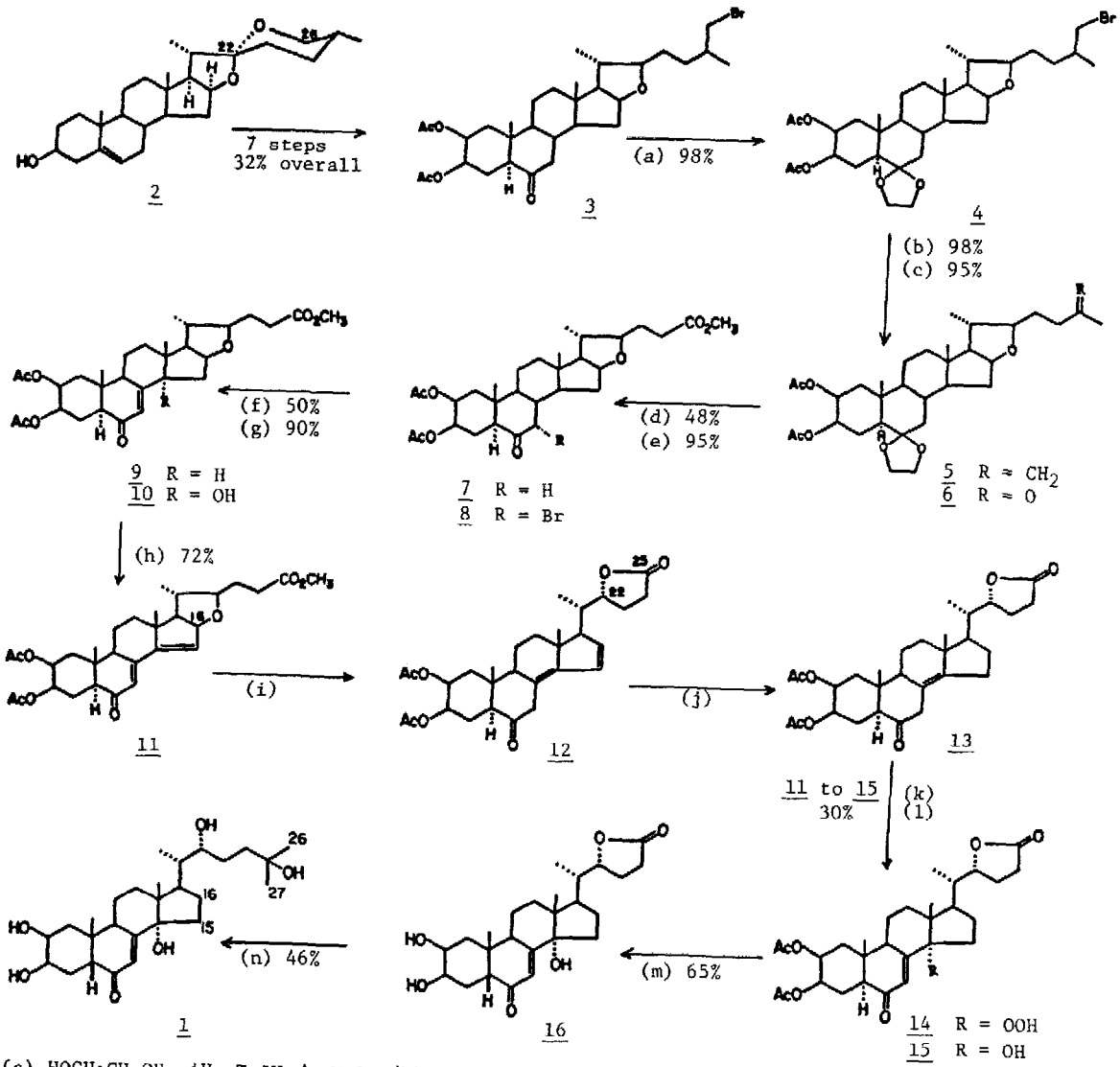

(a) $HOCH_2CH_2OH$, ϕH, TsOH, Δ,$-H_2O$; (b) LiBr, Li_2CO_3, DMF, Δ; (c) O_3, CH_2Cl_2, pyr. (trace), -78°/Zn, AcOH, r.t.; (d) NaOBr, NaOH, H_2O, dioxane/H^+/CH_2N_2, Ac_2O, pyr., r.t., 1 day; (e) Br_2, HOAc, HBr (trace); (f) Li_2CO_3, DMF, Δ; (g) SeO_2, dioxane, 80°; (h) $(CF_3CO)_2O$, pyr., 0°; (i) Zn, AcOH; (j) H_2, Pd-C, EtOAc; (k) O_2, Rose Bengal, MeOH, hv; (l) NaI, MeOH, AcOH (trace); (m) K_2CO_3, MeOH, Δ/H^+; (n) CH_3MgBr, THF.

corresponding 14α-trifluoroacetate, pmr δ 6.11(d,J=3Hz,7-H); ir 1770cm^{-1}. Trace of strong acid equilibrated 11 with the isomeric 9(8),14-dien-6-one, ms 528(M$^+$); pmr δ 5.50(d,J=3Hz,15-H), 4.71 (dd,J=7 and 3Hz,16-H),3.31 and 2.83(ABq,J=22Hz,7-H); uv 245 nm(ε 17,500); cd $\Delta\varepsilon_{295}$ -1.88, $\Delta\varepsilon_{238}$ +3.76; ir 1735, 1720cm^{-1}.

Cleavage of the C-16-O bond was accomplished when a pure sample of 11 was treated with activated zinc dust[5] in degassed boiling acetic acid to yield the desired 8(14), 15-dien-6-one 25↛22 lactone 12, mp 204-205° ($CHCl_3$-ether), ms 498(M$^+$), 85(base peak); pmr δ 6.32 and 5.98 (ABq,J=6Hz, 16/15-H, δ 6.32 peak further split, J=2Hz), 3.29 and 2.90(ABq,J=16Hz,7-H); uv 262nm (ε 15,500), 253nm(ε 16,900); cd $\Delta\varepsilon_{292}$ -6.28,$\Delta\varepsilon_{262}$ +26.1, $\Delta\varepsilon_{255}$ +28.0; ir 1760, 1730, 1720cm^{-1}, a product of concomitant lactonization[6]. Surprisingly, it was later found that the transformation was equally well achieved by simply passing the acetic acid solution of 11 through a short column of activated zinc dust.

Hydrogenation of 12 in ethyl acetate under atmospheric pressure with Pd-C catalyst afforded the desired 8(14)-en-6-one 25↛22 lactone 13, mp 223° (from $CHCl_3$-ether), ms 500(M$^+$); pmr δ 3.10 and 2.85(ABq,J=16Hz,7-H); cd $\Delta\varepsilon_{303}$ -0.41, $\Delta\varepsilon_{203}$ +1.34. Singlet oxygen reacted with 13 in methanol[7] and formed 14α-hydroperoxy-7-en-6-one 25↛22 lactone 14,crystals from acetone-water, gas evolution at 233°; ms 532(M$^+$); pmr δ 5.92(d,J=3Hz,7-H); uv 240 nm(ε 11,500); cd $\Delta\varepsilon_{330}$ +3.44, $\Delta\varepsilon_{244}$ -11.8. Triplet oxygen also reacted with 13 to produce 14, albeit slowly, as 14 was the only contaminant when solutions of 13 were allowed to stand at room temperature for several days.

Standard iodide reduction of 14 yielded 14α-hydroxy-7-en-6-one 25↛22 lactone 15, mp 296-297° (acetone-water), ms 516 (M$^+$), pmr δ 5.91(d,J=3Hz,7-H); uv 239nm(ε 12,000); cd $\Delta\varepsilon_{330}$ +3.13, $\Delta\varepsilon_{245}$ -6.24. The literature procedures[8] were followed for the conversion of 15 to α-ecdysone 1.

To prepare [15,16-$^3H_2$]-α-ecdysone, the labile intermediate 12 in ethyl acetate was tritiated under 1:1 mixture of tritium and hydrogen[9,10]. The crude [^{3}H]-13 was subjected to photooxygenation and sodium iodide reduction affording, after prep-tlc purification, 3.5 mg of [^{3}H]-15 with a specific activity of 18 Ci/mM. It was diluted with 21 mg of cold material to a specific activity of 2.5 Ci/mM (which is sufficiently high for metabolic studies[11,12,13]) and the diluted material was treated with potassium carbonate in refluxing 90% methanol for 2 hours. The desired 5β-product [15,16-$^3H_2$]-16 (1.1 Ci/mM, cd $\Delta\varepsilon_{334}$ +1.4, $\Delta\varepsilon_{244}$ -3.5) was obtained in 65% yield[14] accompanied by the minor 5α isomer (15% yield, cd $\Delta\varepsilon_{334}$ +2.8, $\Delta\varepsilon_{244}$ -7.2). It was noticed that the product retained only half of the original tritium activity, which clearly indicated that some scrambled tritiums were lost during basic hydroysis and isomerization.

Finally, 6.7 mg of [15,16-$^3H_2$]-16 (1.1 Ci/mM in THF was treated with methylmagnesium bromide to afford 3.0 mg of [15,16-$^3H_2$]-α-ecdysone (1.0 Ci/mM after prep-tlc purification).

This procedure provides α-ecdysone 1 from diosgenin 2 in a lower yield (~0.4% overall) than the one reported before[1], but [15,16-$^3H_2$]- and [26,27-$^{14}C_2$]-α-ecdysone (from 16) can be synthesized in a single process utilzing common intermediates with improved stability. [15,16-$^3H_2$]-α-Ecdysone carries tritium labels on the non-labile skeletal C-15/16 positions; it is the

first such labeled ecdysone to be prepared, and together with the side-chain labeled [26,27-$^{14}C_2$]-α-ecdysone should be valuable starting materials for metabolic and physiological studies of insects[15].

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3. Ultraviolet and circular dichroism spectra (JASCO J-40) were taken with methanol as the solvent. Infrared spectra were recorded in $CHCl_3$. Nuclear magnetic resonance spectra were recorded in $CDCl_3$; d=doublet, t=triplet, q=quartet.
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6. The compound 12 decomposed slowly upon standing in solution, but crystals were relatively stable and could be stored.
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9. Tritiation was carried out by New England Nuclear Inc., Boston, Mass.
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